Ectopic Expression of Embryo/Cancer Sequence A (ECSA) in KYSE-30 Cell Line Using Retroviral System

*M Khaleghizadeh [1, 3], MM Forghanifard [2, 3], M Gholamin [3], B Memar[4], MR Abbaszadegan [3]

[1]Department of Microbiology, Damghan Branch, Islamic Azad University, Damghan, Iran. [2]Department of Biology, Damghan Branch, Islamic Azad University, Damghan, Iran.
[3]Human Genetic Division, Immunology Research Center, Avicenna Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran.
[4]Department of Pathology, Imam Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran.

Background
Human preimplantation embryonic cells share many similarities with cancer cells such as ability to self-renew, unlimited proliferation and maintenance of the undifferentiated state. Embryo-cancer sequence A (ECSA), also known as developmental pluripotency associated-2 (DPPA2), is a cancer testis antigen (CTA) with unclear biological function yet. Objective**:** CTAs are expressed normally in germ line cells and trophoblast, and aberrantly in a variety of cancers. According to the importance of ECSA in developmental events and cancer, preparing a suitable platform to analyze its roles seems necessary.

Methods
The coding sequence of the gene was amplified and sub-cloned in pRUF retroviral expression vector. pRUF- ECSA vector was cotransfected with pVSV-G to GP293 cells and with pVSV-G and pGP to HEK293 packaging cell lines. Then the viral particles were transducted to KYSE-30 cells and the concentration of retroviral particles was determined by Real time PCR.

Results
The coding sequence of ECSA gene was successfully subcloned in pRUF expression vector and transfected to packaging cells that the efficiency of transfection to GP293 was higher than the HEK293 cells. The enriched virus particles were obtained at a final concentration of 10^5 TU/ml.

Conclusion
Considering the critical characteristics of retroviral expression system such as stable and longtime expression of interested gene, being safe due to the deletion of retroviral pathogenic genes, and since the function of ECSA gene is not clear, we used this system to induce expression of ECSA and prepared a valuable platform to analyze the biological function of the gene. Also the recombinant ECSA protein can be used in production of recombinant vaccines and serological tests.

Keywords: DPPA2, ECSA, Embryo/cancer gene, KYSE cell line, Retroviral expression system.

Poster Presentation

***Corresponding Author:** M Khaleghizadeh, Human Genetic Division, Immunology Research Center, Avicenna Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran.